**[0016]** FIG. **2**A shows the design of amino acid substitutions in UBS-54 CDR-H3 to fine tune the affinity of EpCAM targeting CARs.

**[0017]** FIG. **2**B shows the recombinant EpCAM binding to MYC-tagged CARs expressed in HEK293T cells. X axis: binding of Alexa Fluor 647 labeled recombinant EpCAM. Y axis: binding of anti-MYC antibody

**[0018]** FIG. **2**C shows the effector to target (E:T) assay for measuring target killing by primary T cells transduced with different EpCAM targeting CARs. Each target was separately incubated with different CAR T cells or non-transduced T (NT) cells at 1:1 E:T ratio. Percent of cytolysis was normalized to luminescence from target cell only.

**[0019]** FIG. **2**D uses Real Time Cell Analyzer (RTCA) to measure primary epithelial cells killing by EpCAM targeting CAR T. Each primary epithelial cell target was separately incubated with CAR T or NT cells at 1:1 E:T ratio. Percent of cytolysis was normalized to target cell only. X axis: Time, Y axis: percent of cytolysis

**[0020]** FIG. **2**E shows the IFN-γ release measured by ELISA for each CAR T variant after co-incubation with different target cells for 24 hours at E:T=1:1.

**[0021]** FIG. **2**F shows the IL-2 release measured by ELISA for each CAR T variant after co-incubation with different target cells for 24 hours at E:T=1:1.

## DETAILED DESCRIPTION OF THE INVENTION

### Definitions

**[0022]** As used herein, "about" refers to ±10% of the recited value.

**[0023]** As used herein, "adoptive T cell therapy" involves the isolation and ex vivo expansion of tumor specific T cells to achieve greater number of T cells than what could be obtained by vaccination alone. The tumor specific T cells are then infused into patients with cancer in an attempt to give their immune system the ability to overwhelm remaining tumor via T cells which can attack and kill cancer.

**[0024]** As used herein, "affinity" is the strength of binding of an antibody (e.g., EpCAM antibody) to its antigen (e.g., EpCAM). Affinity is typically measured and reported by the equilibrium dissociation constant ($K_D$ or Kd), which is used to evaluate and rank order strengths of bimolecular interactions.

**[0025]** As used herein, a "chimeric antigen receptor (CAR)" means a fused protein comprising an extracellular domain capable of binding to an antigen, a hinge domain, a transmembrane domain, and at least one intracellular domain. The receptor is chimeric because they combine both antigen-binding and T-cell activating functions into a single receptor. The "extracellular domain capable of binding to an antigen" means any oligopeptide or polypeptide that can bind to a certain antigen. The "intracellular domain" means any oligopeptide or polypeptide known to function as a domain that transmits a signal to cause activation or inhibition of a biological process in a cell.

**[0026]** As used herein, a "domain" means one region in a polypeptide which is folded into a particular structure independently of other regions.

**[0027]** As used herein, a "single chain variable fragment (scFv)" means a single chain polypeptide derived from an antibody which retains the ability to bind to an antigen. An example of the scFv includes an antibody polypeptide which is formed by a recombinant DNA technique and in which Fv regions of immunoglobulin heavy chain fragment ($V_H$ domain) and light chain fragment ($V_L$ domain) are linked via a spacer sequence. Various methods for engineering an scFv are known to a person skilled in the art. scFv can be in a format of $V_H$-linker-$V_L$ or $V_L$-linker-$V_H$. The linker can be 2-30 amino acids, preferably 5-20 amino acids.

**[0028]** As used herein, a "tumor antigen" means a biological molecule present in tumor and having antigenicity.

### Description

**[0029]** Chimeric antigen receptor (CAR)-T cell therapy has shown robust anti-cancer responses in hematologic malignancies. However, application of CAR-T cell therapeutic approach to solid tumors has been hindered by multiple challenges, one of which is on-target/off-tumor cytotoxicity to normal tissues. Tumor-specific antigens exclusively present on tumor cells are rare. Most CAR-T cells are designed to target tumor associated antigens (TAAs) expressed in high levels on tumor cells. Yet, normal tissues express these antigens as well, albeit at much lower densities.

**[0030]** Epithelial cell adhesion molecule (EpCAM) is highly expressed in epithelial cells and overexpressed in tumor cells in a variety of epithelial carcinomas. High-affinity (nM range) EpCAM-targeting CAR-T cells kill both normal human epithelial cells and EpCAM-high tumor cells in vitro. To mitigate the on-target/off-tumor cytotoxicity, the inventors developed a strategy for fine tuning the affinity of CARs to selectively target tumor cells.

**[0031]** Huls, et al (Nat Biotechnol. 17, 276-281 (1999)) isolated a huMab UBS-54 (UBS-54) that was specific for EpCAM with an affinity of 5 nM. The $V_H$ and $V_L$ sequences of UBS-54 are shown in U.S. Pat. No. 7,777,010, and are incorporated herein by reference. The inventors selected CDR3-$V_H$ of UBS-54 for engineering antibodies with different affinities to EpCAM because CDR3 of $V_H$ occupies a centric position in the antigen binding surface and has the most diversity.

**[0032]** The present invention provides anti-EpCAM antibodies with different affinities to EpCAM. Because the heavy chain variable CDR3 region (CDR-H3) occupies a centric position in the antigen binding surface and has the most diversity[2], the inventors have engineered CDR-H3 for affinity tuning.

**[0033]** The present invention is directed to an antibody or its antigen-binding fragment that binds to EpCAM, wherein the CDR-H3 has the amino acid sequence DPFLHA (SEQ ID NO: 6), DPFLHL (SEQ ID NO: 7), DPFLHV(SEQ ID NO: 8), APFLHY(SEQ ID NO: 3), DPFAHY(SEQ ID NO: 5), or DPFLHF(SEQ ID NO: 9).

**[0034]** In one embodiment, the heavy chain variable CDR1 of the antibody or its antigen-binding fragment has the sequence of GGTFSSY (SEQ ID NO: 10) and the heavy chain variable CDR2 has the sequence of IPIFGT (SEQ ID NO: 11).

**[0035]** In one embodiment, the light chain variable CDR1 of the antibody or its antigen-binding fragment has the sequence of RSSQSLLHSNGYNYLD (SEQ ID NO: 12), the light chain variable CDR2 has the sequence of LGSNRAS (SEQ ID NO: 13), and the light chain variable CDR3 has the sequence of MQALQTFT (SEQ ID NO: 14).